Geographical variation in the leaf volatile oils of *Grindelia ciliata* and *G. adenodonta* (Asteraceae)

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ABSTRACT

The composition of the leaf volatile oils of *Grindelia ciliata* was analyzed and found to be dominated by limonene (31.6 - 43.7%), bornyl acetate (19.2 - 31.6%), α-pinene (14.6 - 23.6%) and β-pinene (8.3 - 12.0%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B. Yields varied from 0.27 to 0.46% (DW). By comparison, the oil of *G. adenodonta* contained limonene (44.6%), bornyl acetate (13.2%), α-pinene (18.0%) and β-pinene (7.4%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B, with a larger yield of 1.39%. Patterns of geographic variation in yields and limonene are presented. Published on-line www.phytologia.org *Phytologia 98(2): 112- 117 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: *Grindelia ciliata, G. adenodonta,* Asteraceae, volatile leaf oil, terpenes, geographic variation, Spanish Gold, giant gumweed.

Grindelia (gumweed) is a large genus of about 75 species with an amphitropical distribution with half of the species occurring in North America and Mexico and the remaining species in South America (Moore, et al., 2012). Steyermark (1934) recognized 45 species, plus 40 varieties and 25 forms (110 taxa). Strother and Wetter (2006) estimated the genus to contain some 30 species; they recognized only 18 species in the Flora of North America. Bartoli and Tortosa (2012) recognized 41 species, 10 varieties and 2 forms in North America based on morphology. Moore et al. (2012) utilized nrDNA and the ETS region as well as psaI-accD cpDNA to analyze selected taxa of *Grindelia* from both North and South America. They found strong support for two sister clades in North and South America. The North American clade seemed to be divided into two groups by the continental divide.

Nesom, Suh and Simpson (1993) submerged the monotypic genus *Prionopsis* (*P. ciliata*) into *Grindelia* as *G. ciliata* (Nutt.l) Spreng. [syn: *G. papposa* (Nutt.) Nesom & Suh]. *Grindelia ciliata* reportedly grows as an annual or biennial. It is widely distributed in Texas, Oklahoma and Kansas, se Colorado, e New Mexico, s Nebraska, s and se Iowa (rare) with putative outlying records from Illinois, Missouri, Arkansas and Louisiana. Previously, we reported on variation in yields of 'bio-crude' (pentane extracts) of *G. ciliata* (Adams et al., 2015).
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Grindelia ciliata is a large plant (up to 2 m), that grows in disturbed sites in various soils and precipitations. It appears to have potential as a semi-arid land bio-crude crop plant. In contrast to most *Grindelia* species, in *G. ciliata*) the leaves and buds are not gummy or with exuded resin, yet, the bio-crude yields are comparable to sticky or gummy *Grindelia* species. *Grindelia adenodonta* (Steyerm.) Nesom is endemic to Texas and grows in the same area as *G. ciliata* near Newcastle - Graham, TX. However, in contrast to *G. ciliata*, it has sticky or gummy leaves. Moore et al. (2012) has shown that these species form a distinct clade as most closely related species. We decided therefore to investigate the volatile leaf oil of *G. adenodonta*. Searches of the literature found no reports on the leaf volatile oils or terpenoids of either *G. ciliata* or *G. adenodonta.*

The purpose of the present paper is to report, for the first time, the composition of the volatile leaf oils of *G. ciliata* and *G. adenodonta*. In addition, we report on geographic variation in the essential oils in natural populations of *G. ciliata*.

MATERIALS AND METHODS

Fresh leaves and specimens of *G. ciliata* were collected from the following populations:

Beav OK, 9 mi N of OK/TX border, on US83, at a ravine on terrace n of Beaver River, mostly single stemmed, locally common on sandy soil. 36° 35' 14" N, 100° 49' 42" W, 2893 ft, 19 Aug 2015, *Adams 14631*, BO1-10,

Cim KS, 3 mi N of KS/OK border on KS Hwy 23, n of Cimarron River on a terrace, locally common, mostly single stemmed, on sandy soil. 37° 01' 27" N, 100° 29' 39.5" W, 2378 ft, 19 Aug 2015, CK1-10C,

Dodge, on US 56, 3 mi w of jct US 56 and US 54, on sandy soil in ditch, Dodge City, KS, scattered but locally common, mostly single stemmed, 37° 43' 13" N, 100° 04' 11" W, 2510 ft, 19 Aug 2015, DCK1-10,

AMR TX, about 17 mi ne of Amarillo, TX, 35° 25' 34" N, 101° 38' 07" W, 3520 ft. on Tex 136, most plants branched. Common on west side of hwy from this location to near Fritch, TX (to last ravine) on Tex 136. in prairie grass, loam soil. 21 Aug 15, FHR1-10,

BOR TX, 1 mi s of Borger, TX on Tex 207 on road cut, sandy but caliche on top, mostly branched plants. 35° 38' 17" N, 101° 23' 50" W, 3203 ft, 22 Aug 2015. *Adams 14636*, BOR1-10,

NewCas, around oil tanks, on red loam, half of the plants were branched, on Bullock Road, near Newcastle TX, 33° 09' 34" N, 98° 41' 54" W, 1217 ft., 30 Aug 2015, *Adams 14642*, BR1-10,

CHD TX, on vacant lot in Childress, red sand, 100s of plants, many branched, on US 287, 34° 24' 47" N, 100° 10' 02" W, 1737 ft, 30 Aug 2015, *Adams 14644*, CHD1-10,

McG, on vacant lot, sandy-loam, ~10 plants, on US84, 7 m n of McGregor, 8 mi. s of Waco, TX, 31° 28' 48" N, 97° 17' 35" W, 540 ft., 27 Aug 2015, *Adams 14641*, MCG 1-6,

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS

The composition of the leaf volatile oils of *Grindelia ciliata* was found (Table 1) to be dominated by limonene (31.6 - 43.7%), bornyl acetate (19.2 - 31.6%), α-pinene (14.6 - 23.6%) and β-pinene (8.3 - 12.0%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B. Yields varied from 0.27 to 0.46% (DW). By comparison, the oil of *G. adenodonta* (Table 1) contained limonene (44.6%), bornyl acetate (13.2%), α-pinene (18.0%) and β-pinene (7.4%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B, with a larger yield of 1.39%. The leaves of *G. ciliata* are not sticky (i.e., with a gummy exudate), whereas the leaves of *G. adenodonta* are covered with exudate and very sticky. It is interesting that these two most closely related species (Moore et al. 2012), also have leaf essential oils that are nearly identical. The oils mostly differ quantitatively except for the presence of some minor components (Table 1).

Because one would expect considerable biochemical changes between leaves and buds, both leaves and buds were collected from a single individual and analyzed separately. Interestingly, the buds volatile oil was lower in yield than leaves (0.32 vs. 0.52%, Table 2). There were also some changes in concentrations (Table 2) between buds and leaves: limonene 43.9, 33,.7%), bornyl acetate (17.8 - 21.4%), α-pinene (15.7, 20.6%) and β-pinene (7.3, 11.3%), borneol (0.4, 1.2%), germacrene D (3.1, 2.2%) and dicyclohexyl-propanedinitrile (0.9, 0.4%).

The yields of volatile oil varied (Fig. 1) from 0.22 (BO Beaver River, OK) and 0.30% (Mcg McGregor, TX) to the largest yields in the High Plains: 0.52% (BO Borger, TX), 0.46% (CK Cimarron River, KS) and 0.45% (AMR Amarillo, TX). Nothing about the habitats at BO and CK indicated that the oils yields would be so different. Both populations were in sandy-loam on south facing slopes with sage.

The % limonene shows (Fig. 2) a different pattern with the highest concentration in DCK (Dodge City, KS) and NC (Newcastle, TX). The remaining populations were fairly uniform in limonene, ranging from 31.6 to 34.3% (Fig. 2).

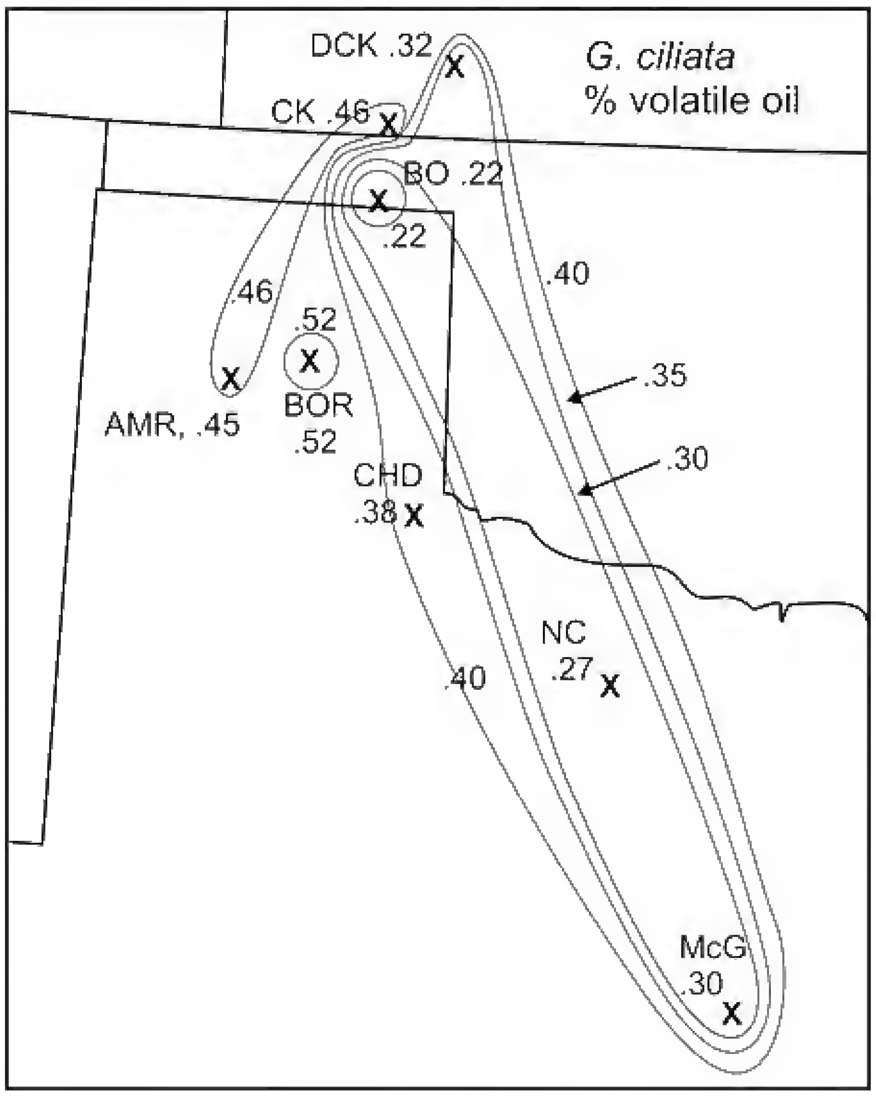

Figure 1. Contour map of yield (% volatile oil).

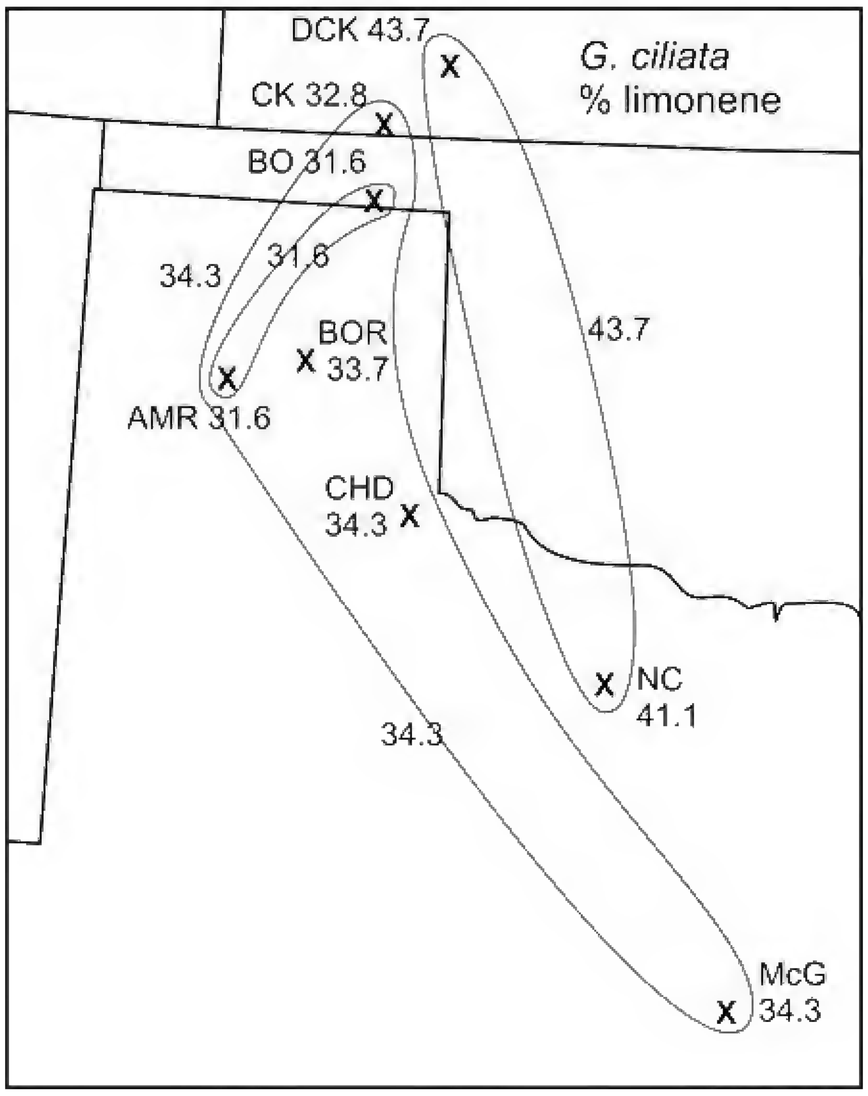

Figure 2. Contour map of % limonene.

ACKNOWLEDGEMENTS

This research supported in part with funds from Baylor University.

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Table 1. Geographical variation in the volatile leaf oils of *Grindelia ciliata*. *Grindelia adenodonta*, Graham, TX, is included for comparison. McG TX = McGregor; TX; NewCas = Newcastle, TX; CHD Tx = Childress, TX; AMR = Amarillo, TX; Bor Tx = Borger, TX; Beav OK = Beaver River, OK; Cim KS = Cimarron River, KS; Dodge = Dodge City, KS. adeno = *G. adenodonta*, Graham, TX. Major components are in bold.

KI	compound	McG Tx 14641	NewCas 14642	CHD Tx 14644	AMR Tx 14635	Bor TX 14636	Beav Ok 14631	Cim Ks 14632	Dodge 14633	adeno 14643
	% yield	**0.30**	**0.27**	**0.38**	**0.45**	**0.52**	**0.22**	**0.46**	**0.34**	**1.39**
921	tricyclene	t	t	t	t	t	t	t	t	t
924	α-thujene	t	t	t	t	t	t	t	t	t
932	**α-pinene**	**17.9**	**14.6**	**19.9**	**20.9**	**20.6**	**15.3**	**23.6**	**15.4**	**18.0**
946	**camphene**	**2.5**	**2.2**	**2.9**	**3.0**	**2.9**	**2.2**	**3.2**	**2.0**	**2.7**
969	sabinene	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3
974	**β-pinene**	**10.9**	**9.4**	**10.4**	**12.0**	**11.3**	**8.3**	**11.3**	**8.0**	**7.4**
988	myrcene	1.4	1.4	1.3	1.2	1.3	1.2	1.4	1.4	1.2
1000	n-decane	t	t	t	t	t	t	t	t	t
1002	α-phellandrene	0.1	t	0.1	t	t	t	t	t	t
1005	o-cresyl methyl ether	t	t	t	t	t	t	t	t	-
1024	**limonene**	**34.3**	**41.1**	**34.3**	**31.6**	**33.7**	**31.6**	**32.8**	**43.7**	**44.6**
1044	**(E)-β-ocimene**	**1.5**	**2.8**	**0.8**	**1.4**	**1.1**	**1.1**	**1.4**	**1.6**	**0.3**
1054	γ-terpinene	0.1	t	0.1	t	t	t	t	t	-
1065	cis-sabinene hydrate	0.2	0.2	0.1	0.2	0.2	t	0.1	t	-
1086	terpinolene	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.2
1100	undecane	0.2	0.2	0.1	0.1	0.2	0.2	t	t	-
1110	octen-3-yl acetate, 1-	t	t	0.1	0.2	t	0.2	t	0.1	-
1122	α-campholenal	-	-	-	-	-	-	-	-	0.2
1135	trans-pinocarveol	-	-	-	-	-	-	-	-	0.3
1140	trans-verbenol	-	-	-	-	-	-	-	-	0.6
1141	camphor	0.3	t	0.2	0.3	0.2	0.3	0.2	t	-
1160	pinocarvone	0.3	0.2	0.1	0.2	0.2	0.2	t	t	0.3
1165	borneol	1.9	2.0	1.1	1.1	1.2	1.8	1.1	0.5	-
1172	cis-pinocamphone	-	-	-	-	-	-	-	-	0.3
1174	terpinen-4-ol	0.2	0.2	0.1	0.2	0.2	0.3	0.4	0.1	0.1
1184	dill ether	t	t	t	t	0.1	t	t	0.1	-
1186	α-terpineol	0.2	0.3	0.2	0.2	0.2	0.2	t	0.2	0.1
1195	myrtenal	0.1	0.1	t	0.1	0.1	0.1	0.1	t	0.1
1195	myrtenol	0.1	0.1	t	0.1	0.1	0.1	0.1	t	0.1
1204	verbenone	-	-	-	-	-	-	-	-	0.2
1215	trans-carveol	-	-	-	-	-	-	-	-	0.1
1239	carvone	-	-	-	-	-	-	-	-	t
1284	**bornyl acetate**	**20.4**	**20.3**	**23.9**	**21.7**	**21.4**	**31.6**	**19.9**	**19.2**	**13.2**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	0.1	-
1453	geranyl acetone	t	0.1	t	t	t	0.2	t	0.2	0.3
1478	γ-muurolene	t	t	t	t	t	t	t	t	-
1480	**germacrene D**	**2.3**	**1.2**	**1.0**	**1.5**	**2.2**	**1.7**	**1.3**	**2.3**	**0.6**
1489	β-selinene	t	t	t	t	t	t	t	t	-
1522	δ-cadinene	t	t	t	t	t	t	t	t	-
1574	germacrene-D-4-ol	t	t	t	t	t	t	t	t	-
1582	caryophyllene oxide	t	t	t	t	t	t	t	t	t
1638	epi-α-cadinol	t	t	t	t	t	0.1	t	t	-
1640	epi-α-muurolol	t	t	t	t	t	0.1	t	t	-
1645	cubenol	t	t	t	t	t	t	t	t	-
1649	**β -eudesmol**	**0.1**	**t**	**t**	**0.3**	**t**	**0.3**	**t**	**t**	**2.9**
1652	α -cadinol	0.1	t	t	t	t	t	t	t	-
1685	germacra-4(15),5, 10(14)-trien-1-al	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.2
1961	propanedinitrile, dicyclohexyl- (NIST)	0.2	0.2	t	0.3	0.4	0.2	0.2	0.9	-
2300	tricosane	t	t	t	t	t	t	t	t	-

Table 2 Comparison of volatile oil from buds and leaves from the same plant from Borger, TX. Components with considerable differences are in bold.

KI	compound	Bor Tx 14627B	Bor TX 14636 L
	% yield	**0.32**	**0.52**
921	tricyclene	t	t
924	α-thujene	t	t
932	**α-pinene**	**15.7**	**20.6**
946	camphene	2.0	2.9
969	sabinene	0.2	0.4
974	**β-pinene**	**7.3**	**11.3**
988	myrcene	1.4	1.3
1000	n-decane	t	t
1002	α-phellandrene	0.2	t
1005	o-cresyl methyl ether	t	t
1024	**limonene**	**43.9**	**33.7**
1044	(E)-β-ocimene	1.4	1.1
1054	γ-terpinene	t	t
1065	cis-sabinene hydrate	t	0.2
1086	terpinolene	0.2	0.2
1100	undecane	t	0.2
1110	octen-3-yl acetate, 1-	t	t
1141	camphor	t	0.2
1160	pinocarvone	t	0.2
1165	**borneol**	**0.4**	**1.2**
1174	terpinen-4-ol	t	0.2
1184	dill ether	0.1	0.1
1186	α-terpineol	t	0.2
1195	myrtenal	t	0.1
1195	myrtenol	t	0.1
1284	**bornyl acetate**	**17.8**	**21.4**
1417	(E)-caryophyllene	0.1	t
1453	geranyl acetone	0.2	t
1478	γ-muurolene	t	t
1480	**germacrene D**	**3.1**	**2.2**
1489	β-selinene	t	t
1522	δ-cadinene	t	t
1574	germacrene-D-4-ol	t	t
1582	caryophyllene oxide	t	t
1638	epi-α-cadinol	0.1	t
1640	epi-α-muurolol	0.1	t
1645	cubenol	t	t
1649	β -eudesmol	t	t
1652	α -cadinol	t	t
1685	germacra-4(15),5, 10(14)-trien-1-al	0.2	0.3
1961	**propanedinitrile, dicyclohexyl- (NIST)**	**0.9**	**0.4**
2300	**tricosane**	**0.4**	**t**